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DNA/BSA binding study of phenothiazine and its *N*-methyl-substituted derivative

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pharmaceuticals MDPI

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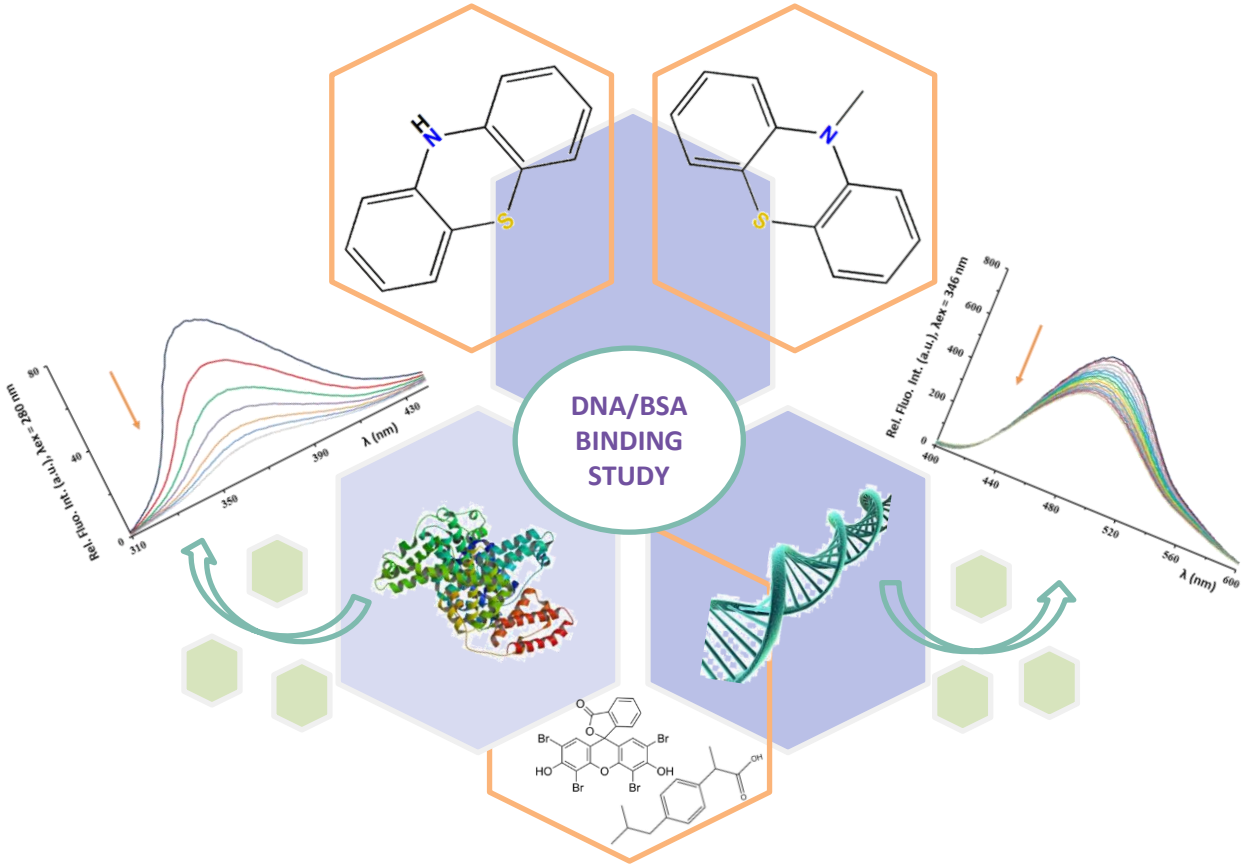


Abstract:

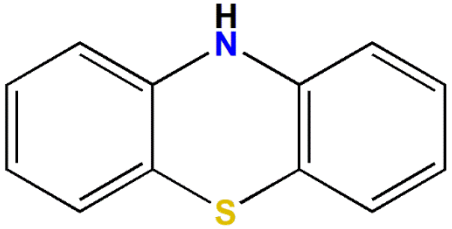
Previous studies have reported that different phenothiazine derivatives have shown a broad spectrum of antibacterial, anticancer and antiplasmid activities. In the present study, we investigated the interactions of phenothiazine (phtz) and its *N*-methyl-substituted derivative, *N*-methylphenothiazine (N-Mephtz) with bovine serum albumin (BSA) and calf thymus DNA (ct-DNA) by fluorescence emission spectroscopy to examine their binding affinity towards these biomolecules. Considering that serum albumin is divided into three domains (I–III), with each domain containing two subdomains (A and B), we have also performed fluorescence competition experiments with site markers for BSA to locate the binding site of the investigated compounds to this biomolecule. Eosin Y was used as a marker for site I (subdomain IIA), while ibuprofen was a marker for site II (subdomain IIIA). The obtained results and the values of binding constants (K_A) have indicated that both phtz and *N*-Mephtz can interact with BSA and ct-DNA, whereby *N*-Mephtz has higher binding affinity towards these biomolecules. On the other hand, K_A values of both investigated compounds are lower in the presence of eosin Y, while only a slight change was observed in the presence of ibuprofen. These results indicated that the binding of the investigated compounds should be mainly located within site I of BSA, and that the tested compounds had to compete with eosin Y to bind to this protein.

Keywords: BSA interaction; DNA interaction; *N*-methylphenothiazine; phenothiazine; site marker

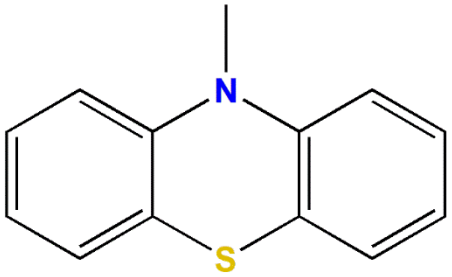
Introduction

- Previous studies have reported that different phenothiazine derivates have shown a broad spectrum of antibacterial, anticancer and antiplasmid activities

- We investigated the interactions of phenothiazine (phtz) and *N*-methylphenothiazine (*N*-phtz) with bovine serum albumin (BSA) and calf thymus DNA (ct-DNA) by fluorescence emission spectroscopy



phenothiazine (phtz)



***N*-methylphenothiazine (*N*-Mephtz)**

Synthesis of *N*-Mephtz

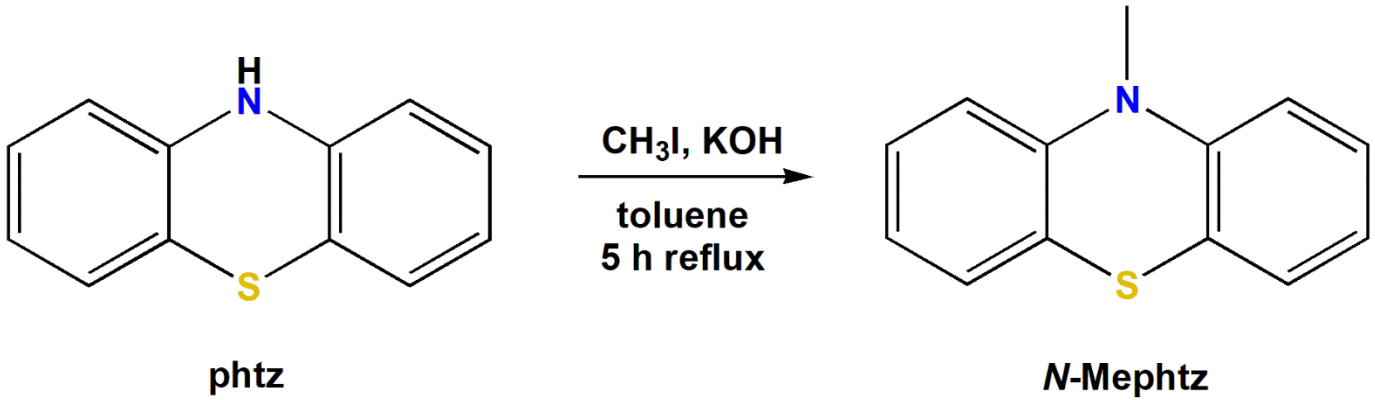


Fig. 1. Schematic presentation of the synthesis of *N*-methylphenothiazine (*N*-Mephtz)

BSA binding study

[*N*-Mephtz] = 0 – 55 µM, PBS (pH = 7.4)

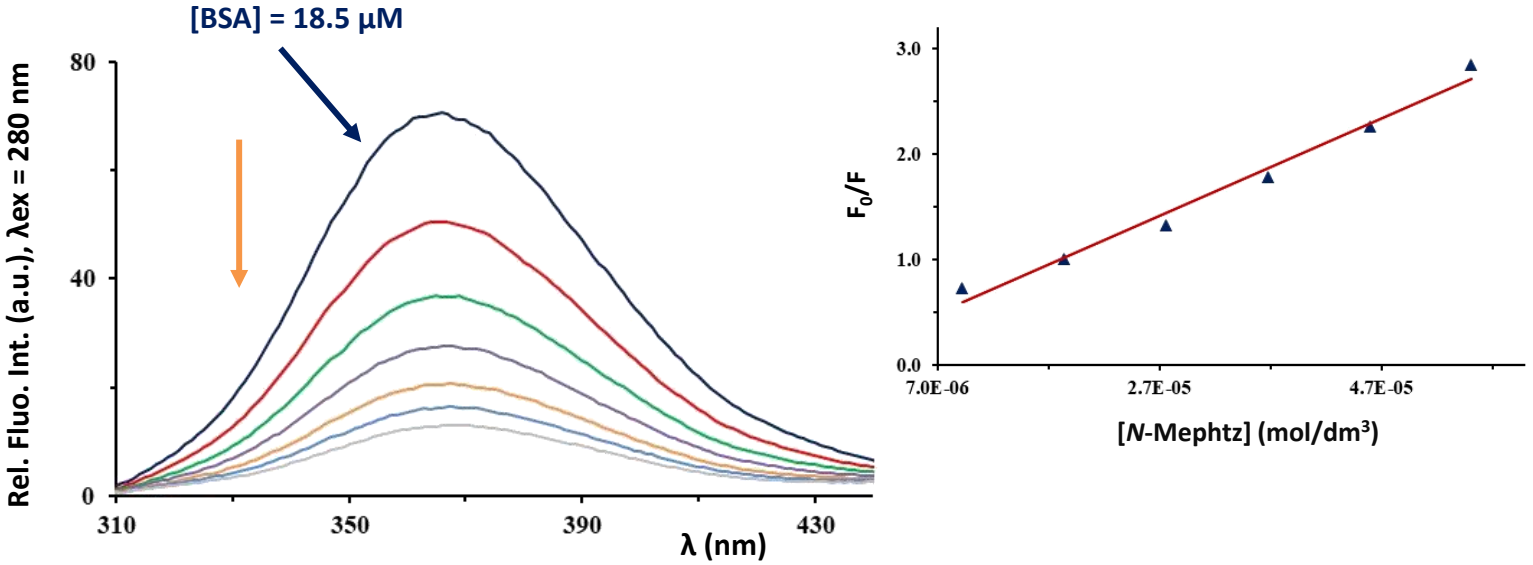
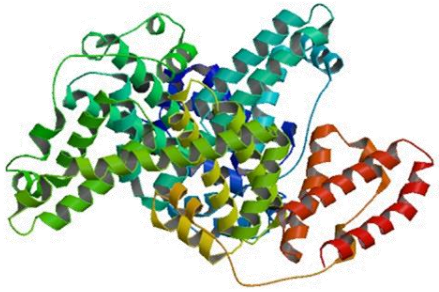


Fig. 2. Fluorescence emission spectra of BSA in the presence of an increasing concentration of *N*-Mephtz. Arrow shows the intensity changes upon increased amount of *N*-Mephtz. Inserted graph: Stern-Volmer plot of F_0/F *vs* [*N*-Mephtz]



BSA binding study

Table 1. Values of the binding constants of phtz and *N*-Mephtz with BSA

	K_{sv} (M^{-1})	Hypochromism (%)	K_q ($M^{-1}s^{-1}$)	K_A (M^{-1})	*n*
phtz	$(3.65 \pm 0.02) \times 10^4$	70.5	3.65×10^{12}	1.36×10^5	1.14
N-Mephtz	$(2.69 \pm 0.11) \times 10^5$	74.4	2.69×10^{13}	2.43×10^6	1.35

Competitive experiments with BSA and site markers

[*N*-Mephtz] = 0 – 35 µM, PBS (pH = 7.4)

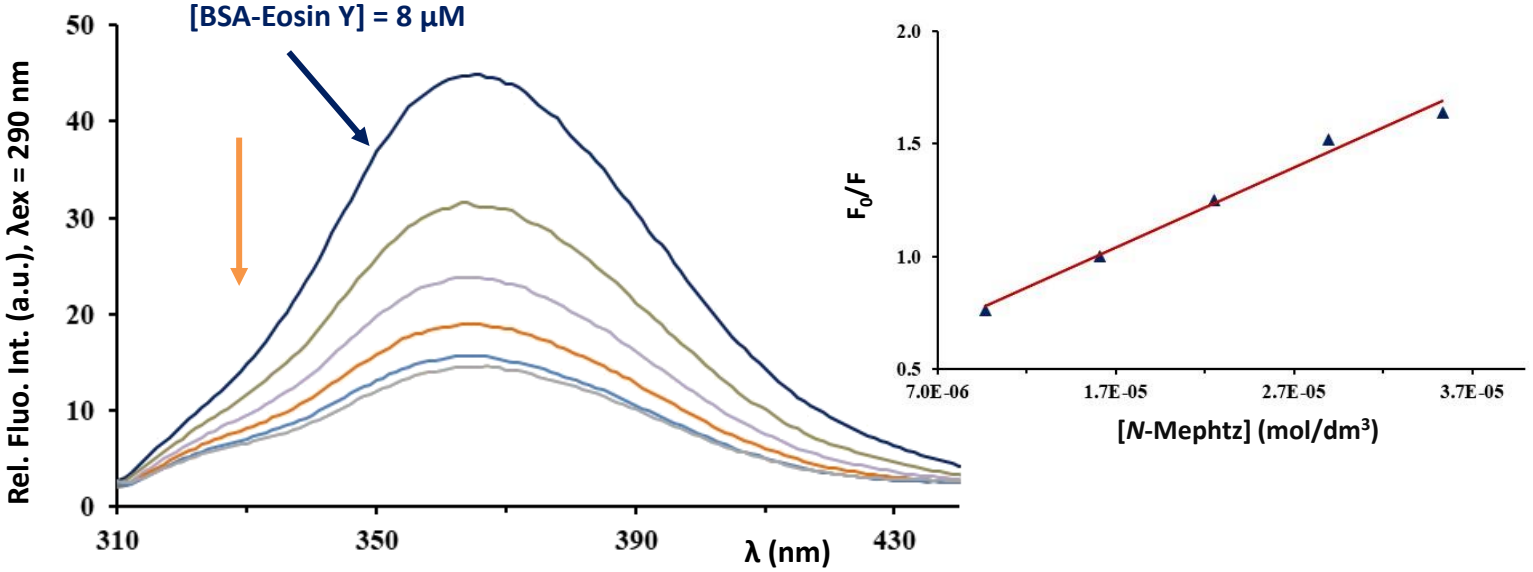


Fig. 3. Fluorescence emission spectra of BSA-Eosin Y in the presence of an increasing concentration of *N*-Mephtz. Arrow shows the intensity changes upon increased amount of *N*-Mephtz. Inserted graph: Stern-Volmer plot of F_0/F *vs* [*N*-Mephtz]

Competitive experiments with BSA and site markers

Table 2. Values of the binding constants of phtz to BSA in absence and in presence of the site markers, eosin Y and ibuprofen

	K_{sv} (M^{-1})	Hypochromism (%)	K_q ($M^{-1}s^{-1}$)	K_A (M^{-1})	*n*
phtz	$(3.65 \pm 0.02) \times 10^4$	70.5	3.65×10^{12}	1.36×10^5	1.14
Ibuprofen	$(2.02 \pm 0.01) \times 10^4$	67.6	2.02×10^{12}	3.21×10^4	1.05
Eosin Y	$(1.66 \pm 0.03) \times 10^4$	64.2	1.66×10^{12}	2.16×10^4	1.03

Competitive experiments with BSA and site markers

Table 3. Values of the binding constants of *N*-Mephtz to BSA in absence and in presence of the site markers, eosin Y and ibuprofen

	K_{sv} (M^{-1})	Hypochromism (%)	K_q ($M^{-1}s^{-1}$)	K_A (M^{-1})	*n*
***N*-Mephtz**	$(2.69 \pm 0.11) \times 10^5$	74.4	2.69×10^{13}	2.43×10^6	1.35
Ibuprofen	$(1.37 \pm 0.03) \times 10^5$	70.9	1.37×10^{13}	3.33×10^6	1.38
Eosin Y	$(8.12 \pm 0.06) \times 10^4$	67.8	8.12×10^{12}	7.20×10^5	1.23

DNA binding study

[*N*-Mephtz] = 0 – 135 µM, PBS (pH = 7.4)

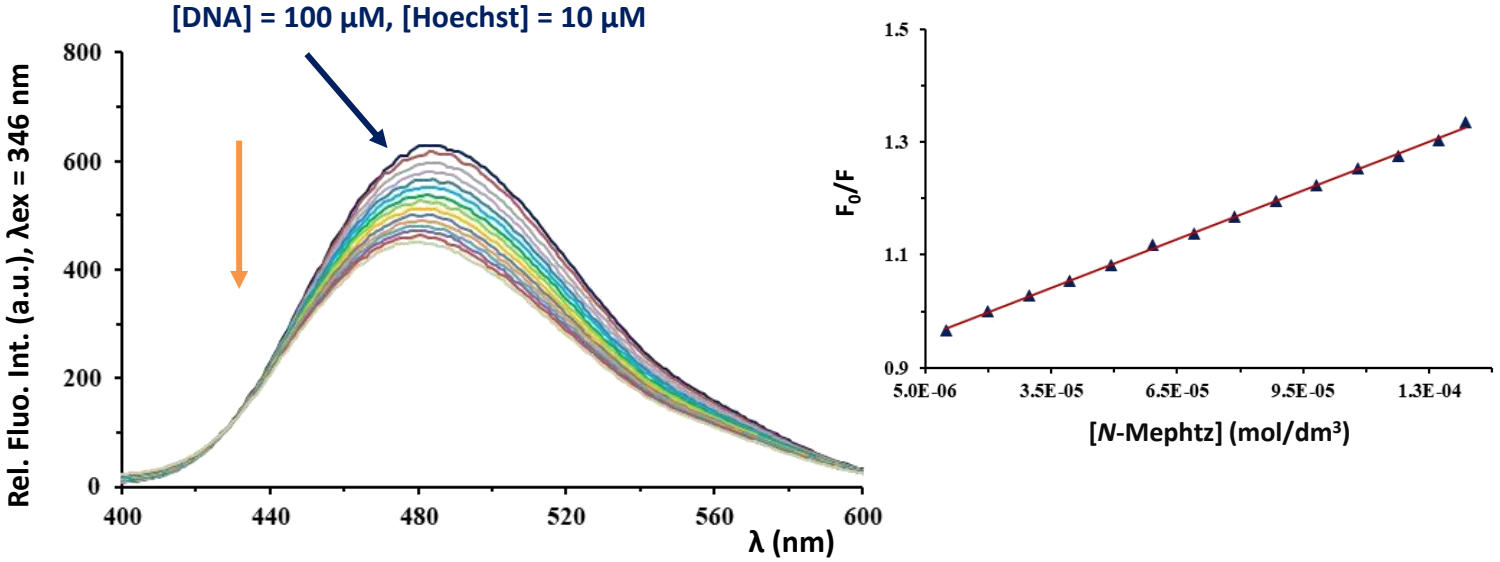


Fig. 4. Fluorescence emission spectra of ct-DNA-Hoechst 33258 system in the presence of an increasing concentration of *N*-Mephtz. Arrow shows the intensity changes upon increased amount of *N*-Mephtz. Inserted graph: Stern-Volmer plot of F_0/F *vs* [*N*-Mephtz]

DNA binding study

Table 4. Values of the binding constants of phtz and *N*-Mephtz with ct-DNA-Hoechst 33258 system

	K_{sv} (M^{-1})	Hypochromism (%)	K_q ($M^{-1}s^{-1}$)	K_A (M^{-1})	*n*
phtz	(1.96 ± 0.003) x 10^3	20.4	1.96 x 10^{11}	3.08 x 10^2	0.79
N-Mephtz	(3.07 ± 0.002) x 10^3	29.2	3.07 x 10^{11}	6.12 x 10^3	1.08

Conclusions

- The obtained results have indicated that both phtz and *N*-Mephtz can interact with BSA and ct-DNA, whereby *N*-Mephtz has higher binding affinity towards these biomolecules
- K_A values of both investigated compounds are lower in the presence of eosin Y, while only a slight change was observed in the presence of ibuprofen
- The binding of the investigated compounds should be mainly located within site I of BSA

Acknowledgments

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